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Docket No.: M0656.70097US00
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Alice Y. Ting
Serial No.:	10/634,740
Confirmation No.:	8302
Filed:	August 5, 2003
Patent No.:	7,056,683
For:	GENETICALLY ENCODED FLUORESCENT REPORTERS OF KINASE, METHYLTRANSFERASE, AND ACETYL-TRANSFERASE ACTIVITIES
Examiner:	R. B. Mondesi
Art Unit:	1652

Certificate of Mailing Under 37 CFR 1.8(a)

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Attention: Certificate of Correction Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: August 6, 2007

MaryDilys S. Anderson, Ph.D., Registration No. 52,560

REQUEST FOR CERTIFICATE OF CORRECTION
PURSUANT TO 37 CFR 1.323

Attention: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Upon reviewing the above-identified patent, Patentee noted an error, which should be corrected.

In the Specification: At column 1, lines 12-16, the Government Support section should be updated to read:

GOVERNMENT SUPPORT

This invention was made with government support awarded by the U.S. Navy under Grant Number N00014-03-1-0456 and by the National Institutes of Health under Grant Number 5-K22-HG002671-02. The government has certain rights in the invention.

The error was found in the application as filed by applicant. Our check in the amount of $100.00 covering the fee set forth in 37 CFR 1.20(a) is enclosed.

The error now sought to be corrected is an inadvertent error the correction of which does not involve new matter or require reexamination.

Transmitted herewith is a proposed Certificate of Correction effecting such amendment. Patentee respectfully solicits the granting of the requested Certificate of Correction.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 23/2825, under Docket No. M0656.70097US00. A duplicate copy of this paper is enclosed.

Dated: August 6, 2007

Respectfully submitted,

By _____
MaryDilys S. Anderson, Ph.D.
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PTO/SB/44 (07-07)
Approved for use through 07/31/2007. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	7,056,683
APPLICATION NO.	:	10/634,740
ISSUE DATE	:	June 6, 2006
INVENTOR(S)	:	Alice Y. Ting

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the specification, column 1, lines 12-16 the Government Support section should read as follows:

GOVERNMENT SUPPORT

This invention was made with government support awarded by the U.S. Navy under Grant Number N00014-03-1-0456 and by the National Institutes of Health under Grant Number 5-K22-HG002671-02. The government has certain rights in the invention.

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Dated: August 6, 2007 Signature: _____ (MaryDilys S. Anderson, Ph.D.)

MAILING ADDRESS OF SENDER (Please do not use customer number below):
MaryDilys S. Anderson, Ph.D.
WOLF, GREENFIELD & SACKS, P.C.
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Docket No.: M0656.70097US00
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Dated: August 6, 2007

MaryDilys S. Anderson, Ph.D., Registration No. 52,560

TRANSMITTAL LETTER

Attention: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Enclosed are the following items for filing in connection with the above-referenced Patent:

1. Request for Certificate of Correction
2. Certificate of Correction
3. Copy of Pertinent Page from US Patent No.: 7,056,683

Our check in the amount of $100.00 covering the required fee is enclosed. The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 23/2825, under Docket No. M0656.70097US00. A duplicate copy of this paper is enclosed.

Dated: August 6, 2007

Respectfully submitted,

By _____
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1230675.1

GENETICALLY ENCODED FLUORESCENT REPORTERS OF KINASE, METHYLTRANSFERASE, AND ACETYL-TRANSFERASE ACTIVITIES

RELATED APPLICATIONS

This application claims priority under 35 U.S.C. §119 to U.S. provisional application Ser. No. 60/425,578, filed Nov. 12, 2002.

GOVERNMENT SUPPORT

This invention was made with government support under Grant Number N00014-03-1-0456 awarded by the Navy. The government has certain rights in the invention.

FIELD OF THE INVENTION

The invention relates to products and methods of making and using fusion protein reporters to monitor histone modification, such as acetylation, methylation, and phosphorylation. The invention relates to the use of fusion protein reporters in methods and compositions for the diagnosis and evaluation of histone modification-associated disorders.

BACKGROUND OF THE INVENTION

Recent advances in protein engineering, chemistry, and fluorescence microscopy have resulted in improved quantitative reporters of signaling events in living cells (Zhang, J. et al., *Nat. Rev. Mol. Cell. Biol.*, 2002, 3(12):906–18). For example, the engineering of spectrum-altered fluorescent proteins (FPs) from the *Aequorea victoria* Green Fluorescent Protein (GFP) has enabled simultaneous real-time measurement of multiple protein expression and localization patterns in live cells. FP-based indicators are less toxic than simple organic dyes and can respond to a wider range of biological events; they can also be targeted to subcellular compartments through genetic fusion and can be introduced into a wider variety of tissues and into intact organisms.

Although great strides have been made in FP-based indicator development, there are drawbacks in the existing technology. Existing indicators have been designed on a "custom cut", one-at-a-time basis. They are thus currently capable of reporting only a handful of the thousands of cellular signaling state variables. Additionally, few existing FP indicators have been developed to report on the more complex cellular parameters such as enzyme activity. These "hidden" variables are implicated in every known signaling pathway, but their direct observation has not been effectively addressed by current methodology.

SUMMARY OF THE INVENTION

The invention includes fusion protein reporters that may be used to monitor the modification of proteins (e.g. histones) in cells, including real-time monitoring of protein (e.g. histone) modification in living cells. Use of these novel reporters allows the determination of the level of modification of proteins, for example histones, including the level of acetylation, methylation, and phosphorylation. These determinations can be compared to control levels and thus allow diagnosis of disorders that are associated with levels of protein (e.g. histone) modification that differ from normal levels. The reporters can also be used to evaluate candidate pharmaceutical agents for use in prevention and/or treatment of protein modification-associated disorders, for example histone-modification-associated disorders. These evaluations can be done in cells, tissues, samples, or in subjects to determine the effect of pharmaceutical agents on the level of protein (e.g. histone modification. In addition, the fusion protein reporters of the invention can be utilized in non-invasive methods to assess cellular response to external stimuli.

According to one aspect of the invention, fusion protein reporters are provided. The fusion proteins include a core comprising a histone-modification-specific binding domain conjugated to a histone polypeptide substrate, wherein the core is flanked by donor and acceptor fluorescent moieties. In some embodiments, the histone modification specific binding domain is conjugated to the histone polypeptide substrate with a linker molecule. In certain embodiments, the fusion protein reporter also includes one or more additional histone-modification-specific binding domains. In some embodiments, the histone polypeptide substrate is selected from the group consisting of H3 or H4. In some embodiments, the histone polypeptide is selected from the group consisting of the N-terminus of H3 and the N-terminus of H4. In some embodiments, the donor fluorescent moiety is selected from the group consisting of cyan fluorescent protein (CFP), enhanced cyan fluorescent protein (ECFP), and the A206K mutants of these proteins (non-dimerizing). In certain embodiments, the acceptor fluorescent moiety is selected from the group consisting of yellow fluorescent protein (YFP), enhanced yellow fluorescent protein (EYFP), Citrine, and Venus, and the A206K mutants of these proteins. In some embodiments, the histone modification is selected from the group consisting of acetylation, methylation, and phosphorylation. In some embodiments, the histone modification-specific binding domain is selected from the group consisting of: 14-3-3, FHA, WW, bromodomain, and chromodomain. In some embodiments, the bromodomain is selected from the group consisting of: Gcn5, TAF$_{II}$250, P/CAF, CBP, BRG1, Swi2, and Sth1. In some embodiments, the chromodomain is selected from the group consisting of: HP1, MRG15, MRG-1, cynCDY, Hrp3, dMi-2, CHD5, Swi6, and pdd3p. In certain embodiments, the bromodomain comprises the amino acid sequence set forth as SEQ ID NO: 3. In some embodiments, the histone polypeptide is a polypeptide substrate for the histone-modification-specific binding domain. In certain embodiments, the histone polypeptide is an H3 polypeptide comprising the amino acid sequence set forth as ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHR (SEQ ID NO:1). In certain embodiments, the histone polypeptide is an H3 polypeptide comprising the amino acid sequence set forth as ARTKQTARKSTGGKAPRKQLATKAARKSAP (SEQ ID NO: 18). In other embodiments, the histone polypeptide is an H4 polypeptide comprising the amino acid sequence set forth as SGRGKGGKGLGKGGAKRHRKVLRDNIQGIT (SEQ ID NO:2). In certain embodiments, the fusion protein reporter also includes a targeting polypeptide, associated with the fusion protein. In some embodiments, the targeting polypeptide is selected from the group consisting of a receptor ligand and a nuclear localization sequence (NLS), nuclear export signal (NES), plasma membrane targeting signal, a histone binding protein, a histone protein and a nuclear protein.

In some aspects, the invention provides expression vectors comprising an expression cassette encoding a fusion protein reporter of any of the forgoing embodiments. Some aspects of the invention also provide host cell transformed or transfected with the expression vector.